Original Article

Diagnostic utility of PAX8 in differentiation of mullerian from non-mullerian tumors

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Abstract

Background: Considering the high prevalence of female genital tract neoplasms, non-specific nature of the initial symptoms, higher possibility of metastasis by the time of diagnosis, importance of differentiating metastatic Mullerian tumors or metastatic breast cancer in the female genital tract, especially in the ovary, and lack of diagnostic markers with high sensitivity and specificity, the purpose of the current study was to evaluate the utility of Paired box protein8 (PAX8) expression in Mullerian and non-Mullerian neoplasms.

Materials and Methods: In this descriptive–analytic, cross-sectional study, paraffin-embedded tissues of patients with definitive pathologic diagnosis of Mullerian and non-Mullerian tumors were selected. PAX8 immunohistochemical (IHC) staining was performed for all selected blocks. Immunopositivity of the slides for PAX8 was reviewed. It was defined as the presence of nuclear staining in at least 10% of the tumor cell nuclei.

Results: Thirty-seven Mullerian (including 18 ovarian epithelial tumors, 17 endometrial carcinoma and two endocervical adenocarcinoma) and 37 non-Mullerian tumors were studied for PAX8 expression. Twenty-nine of 37 (78.4%) and one of 37 (2.7%) of the Mullerian and non-Mullerian tumors were positive for PAX8, respectively. The sensitivity and specificity of PAX8 by IHC for differentiation of Mullerian from non-Mullerian tumors was 78.4% and 97.3%, respectively.

Conclusion: Our findings indicated that PAX8 could be used as a useful IHC marker for diagnosing Mullerian tumors. It has moderate to high sensitivity, but high specificity, for diagnosing carcinomas of Mullerian origin.

Key Words: Female, genital tract, immunohistochemical staining, mullerian, PAX8, tumor

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INTRODUCTION

Neoplasms of the female genital tract are considered as the third most common malignancy in women, with a high rate of morbidity and mortality.[1] These groups of tumors are mostly diagnosed in an advanced stage of the disease and, therefore, the survival rate is low. On the other hand, pathologic features of these tumors are similar to other tumors, especially breast metastatic cancers. Differentiating metastatic female genital tract tumors, especially ovarian cancers from breast cancer or metastatic breast cancer that metastase to ovary, from primary ovarian tumor is one of the most important issues in this field.[2] It seems that an appropriate tumor staging and management could be achieved only if the primary and metastatic type of the tumor was determined properly.

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Many immunohistochemical (IHC) markers are identified to determine the primary origin of the tumors, but most of them have relatively low sensitivity and specificity. Regarding the carcinomas of Mullerian origin, few specific IHC markers have been identified. Markers such as CA-125, ER, PR and WT-1 are often used in combination with other IHC markers to determine the possible Mullerian origin and the primary type of the tumors,[3-5] but they have limited value in this field because of low sensitivity and specificity.

Recent evidences support the utility of PAX8 factor in this regard, especially in determining Mullerian tumors.[6-8] Differentiation of ovarian metastatic tumors (especially with breast origin) from primary ovarian tumors can also be performed based on this IHC staining.

PAX8 is a member of the paired-box family of genes. It is expressed during organogenesis of the thyroid gland, Mullerian tract and kidney. The expression of PAX8 has been reported in renal, thyroid and ovarian neoplasms.[9,10] Recent studies reported that PAX8 was considered as a surrogate marker for carcinoma of Mullerian origin.[11,12]

Considering the high prevalence of female genital tract neoplasms, non-specific nature of the initial symptoms, higher possibility of metastasis by the time of diagnosis and lack of diagnostic markers with high sensitivity and specificity, the purpose of the current study was to evaluate the utility of IHC PAX8 expression in Mullerian and non-Mullerian neoplasms.

MATERIALS AND METHODS

In this descriptive–analytic, cross-sectional study, paraffin-embedded tissues of 37 patients with definitive pathologic diagnosis of epithelial tumors of ovary, uterus and endocervical adenocarcinomas (Mullerian tumors) and 37 tissue sections from patients with definite diagnosis of non-Mullerian tumors (except for renal and thyroid) were retrieved from the pathology archives of Alzahra Hospital in Isfahan-Iran, from March 2008 to March 2010. All sections were selected by the simple sampling method.

The Medical Ethics Committee of the Isfahan University of Medical Sciences approved the study protocol (research project number; 390098).

PAX8 IHC staining was performed for all selected blocks. IHC-stained slides were prepared for all specimens. The slides were evaluated by a pathologist using light microscopy. Immunopositivity for PAX8 was defined as the presence of nuclear staining in at least 10% of the tumor cell nuclei.

PAX8 immunohistochemistry staining method

PAX8 IHC staining was done manually according to the proposed manufacturer protocol (abcam-Ab53490-UK) with the mouse polyclonal antibody.

In brief, 4-μm-thick paraffin-embedded sections were made and processed on the poly-L-lysine-coated slides. These slides were sunk in xylene and then in alcohol and finally in Tris-EDTA buffer with a pH of nearly 9 (at a temperature of 95°C and for 20 min) for deparafinazation and antigen retrieval. The next steps for staining were as follows:

1. Incubation in 3% hydrogen peroxide for 10 min
2. Incubation in mouse anti-PAX8 polyclonal antibody (1/200 dilution) for 25 min
3. Coating the slides with polymer EnVision for 20 min
4. Incubation in diaminobenzidine (DAB) chromogen substrate solution for 5 min
5. Counter staining with hematoxylin.

Positive controls included normal endometrium. Negative controls were obtained by omitting the primary monoclonal antibody.

Statistical analysis

The data obtained were analysed using SPSS version 17 for windows software.

RESULTS

In this study, 37 Mullerian tumors, including 18 ovarian epithelial tumors (11 papillary serous carcinoma, three mucinous carcinoma, two endometrioid carcinoma, two clear-cell carcinoma), 15 endometrioid carcinoma of uterine corpus, one malignant mixed Mullerian tumor of uterine corpus, one papillary serous carcinoma of uterine and two endocervical adenocarcinoma and 37 non-Mullerian tumors, including 14 breast carcinoma, seven colon, five gastroesophagial, five lung, three pancreas and three bladder carcinomas were retrieved from the pathology archives of the Alzahra Hospital.

Expression of PAX8, by IHC, in Mullerian and non-Mullerian tumors is presented in Table 1 and Figures 1-3.

In total, in 29/37 tumors from the Mullerian tumors and in one of 37 tumors from the non-Mullerian tumors, the expression of PAX8 was positive. The sensitivity and specificity of PAX8 by IHC for differentiation of Mullerian from non-Mullerian tumors was 78.4% and 97.3%, respectively.

Table 1: Expression of PAX8 in Mullerian and non-Mullerian tumors

Primary site and histology type	Case number	Positive PAX8 staining by IHC (%)
Mullerian tumors		
-Ovary		
Papillary serous carcinoma	11	9 (81.8)
Mucinous carcinoma	3	0 (0)
Endometrioid carcinoma	2	2 (100)
Clear-cell carcinoma	2	2 (100)
-Uterine corpus		
Endometrioid carcinoma	15	14 (93.3)
Malignant mixed Mullerian tumor	1	1 (100)
Papillary serous carcinoma	1	1 (100)
-Cervix		
Endocervical adenocarcinoma	2	0 (0)
Non-mullerian tumors		
-Breast	14	0 (0)
-Colon	7	0 (0)
-Gastroesophagial	5	0 (0)
-Lung	5	0 (0)
-Bladder	3	1 (33.3)
-Pancreas	3	0 (0)

DISCUSSION

In this study, the expression of PAX8 was determined in Mullerian and non-Mullerian tumors. Our findings indicated that PAX8 could be used as a useful IHC marker for the diagnosis Mullerian tumors with acceptable sensitivity and specificity.

The expressions of transcription factors including PAX8 have been reported in female genital tract neoplasms in recent studies. Tacha *et al*. have investigated the IHC expression of PAX8 in 1100 cases of normal and neoplastic tissues. They indicated that in ovarian cancers, PAX8 stained 79% (181/229) of all ovarian carcinomas. PAX8 was positive in 92% (101/109) of serous adenocarcinomas, 83% (49/59) of endometrioid carcinomas, 100% (3/3) of clear cell carcinomas and 50% (27/54) of mucinous cystadenocarcinomas. They concluded that it could be a useful marker in a histopathology laboratory setting for diagnosing the primary type of female genital tract tumors.[13]

Ozcan and colleagues in a comprehensive IHC study have studied the expression of PAX8 in non-neoplastic tissues, primary tumors and metastatic tumors. According to their findings, 238/267 (89%) Mullerian-type neoplasms were positive for PAX8, and they concluded that PAX8 is a sensitive and specific marker for both primary and metastatic renal, Mullerian and thyroid tumors.[14]

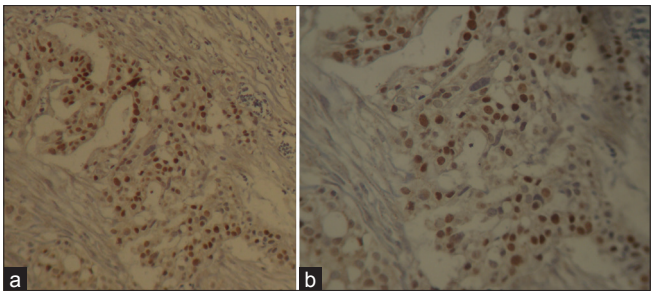

Figure 1: (a) Ovarian serous adenocarcinoma demonstrating PAX8 nuclear staining (×100) (b) Ovarian serous adenocarcinoma demonstrating PAX8 nuclear staining (×400)

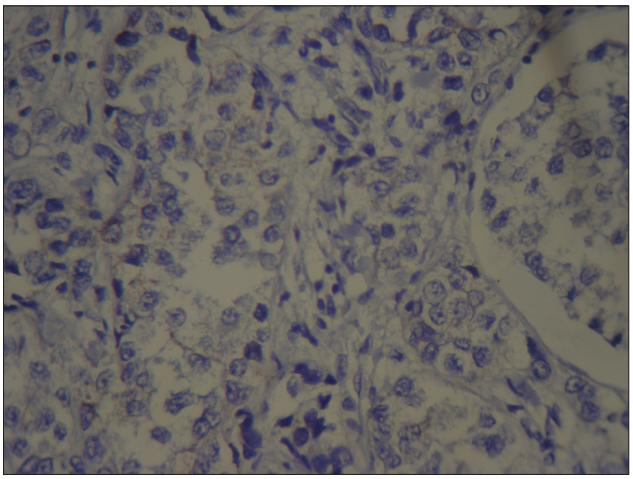

Figure 2: Invasive breast carcinoma with negative PAX8 staining

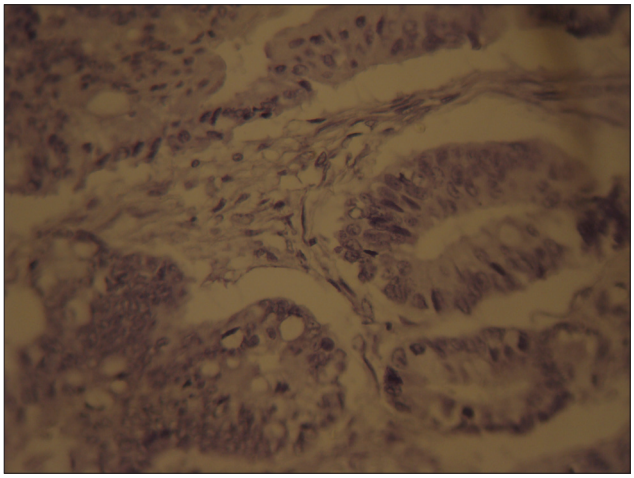

Figure 3: Colon carcinoma with negative PAX8 staining

In this study, we investigated the IHC expression of PAX8 in determining the type and origin of the primary tumors. The utility of IHC in this field has been mentioned in previous studies.[15]

Recently, PAX8 expression in the Mullerian system has received considerable interest in the clinical and research fields. Many studies have reported positive expression of PAX8 in fallopian tube secretory cells,

in distinguishing serous ovarian carcinoma from breast carcinoma and many ovarian/pelvic serous tumors.[11,12,16] Some other studies have reported its expression in non-serous ovarian tumors, the endometrium and also the cervix.[17]

In this study, PAX8 was positive in the mentioned tumors and was negative in mucinous ovarian carcinomas and endocervical adenocarcinoma. In accordance to our study, Laury *et al*. have reported relatively low expression (40%) of PAX8 in primary mucinous ovarian carcinomas.[17] Lower expression of PAX8 in endocervical adenocarcinoma has been reported in other studies also. Tong and colleagues have reported similar results regarding mucinous ovarian carcinomas and endocervical adenocarcinoma.[8] However, the absence of PAX8 does not mean that the tumor is not ovarian or Mullerian in origin. The causes of such findings need further investigations.

In accordance with the study of Tong *et al*., in all types of the studied uterine carcinomas, PAX8 expression were positive, which may be related to the diffuse positive PAX8 staining in the normal endometrium.

Overall, these findings confirm the suggestions of previous studies regarding the sensitivity of this marker. The results of this study were similar to those of Tong *et al*. and Bowen *et al*.[8,11]

In this study, we determined the specificity of PAX8 by investigating its expression in non-Mullerian tumors. It was positive only in one case with bladder cancer. Our results showed that PAX8 was negative in 97.3% of non-Mullerian tumors (except for thyroid and renal carcinomas), which indicates its higher specificity in this regard. Our results were consistent with the reports of Wiseman *et al*. and Nonaka *et al*.[7,11] It seems that a better conclusion would be achieved if we had studied a wider variety of non-Mullerian tumors.

In summary, PAX8 could be used as a useful IHC marker for diagnosing Mullerian tumors (except for mucinous adenocarcinoma of the ovary and adenocarcinoma of the cervix). It has moderate to high sensitivity, but a high specificity, for diagnosing carcinomas of Mullerian origin.

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